ENDOCRINE FUNCTIONS OF THE PANCREAS

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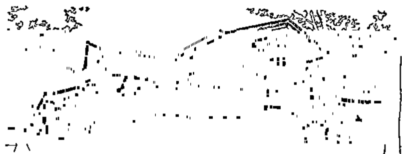
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TO MY FATHER

Skillful surgeon, original thinker, wise counselor

PREFACE

As in every field of medicine, the surgical and medical treatment of diseases of the pancreas has advanced to a point from which further progress is dictated almost entirely by the basic contributions of physiologists and biochemists. It was in view of this that it seemed worthwhile to collect such past and recent physiological information as might be pertinent to the further understanding of clinical situations in which the pancreas plays a part. The experimental literature dealing with the pancreas as an endocrine organ is vast, and although a considerable amount of bibliographic material has been cited, this review is far from exhaustive. The bibliography included here must be considered only an introduction to this absorbing field of scientific literature.

Many of the most significant questions considered here have been the basis of heated divergences of scientific opinion. Some of these controversies, which have lasted for decades, remain unsettled at the present time. In dealing with these problems, I have tried to present some of the data on each side of the arguments Where final conclusions can not yet be reached, the experimental facts, at least, should be known to us

The book would be incomplete without the expression of my profound gratitude to four men with whom it has been my privilege to be associated during the past seven years and who have made it possible for me to pursue my interest in the pancreas. They are Dr Edwin B. Astwood of Boston, Captain Albert R Behnke and the late Captain Erik G Hakansson of the Naval Medical Research Institute, and Dr. Owen H. Wangensteen of the University of Minnesota

I am indebted to Dr. Gerald T. Evans for reading and criticizing the text

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I am grateful to Mrs. Marion Jacobson and her staff, to my mother, and particularly to my wife Elizabeth for their help in the preparation of the manuscript.

I wish also to acknowledge the kindness of the publishers of the *Journal of Biological Chemistry, American Journal of Physiology, Endocrinology,* and *Archives Internationales de Physiologie* for permission to reproduce figures from these journals.

BERNARD ZIMMERMAN

CHAPTER IV

ENDOCRINE FUNCTIONS
OF THE
PANCREAS

CHAPTER I

HISTORY

THE PANCREAS AS AN ORGAN OF INTERNAL SECRETION

THE first suggestion that diabetes mellitus might be related to the impairment of some function of the pancreas is believed to be that contained in a case report written by Thomas Cawley in 1788 (11). In this interesting and thoughtful report the author described a case of severe, rapidly progressive diabetes which terminated in characteristic diabetic coma. An autopsy disclosed extensive pancreatic lithiasis with sclerosis and atrophy of the pancreatic tissue. Cawley reviewed the theories of diabetes which existed at his time. There were two general schools: one which believed the liver was the organ primarily affected in diabetes and a second which held that the kidneys were the seat of the disorder. After discussing these theories Cawley concluded that neither applied to his case and suggested that the disease of the pancreas in this instance was the primary pathologic process which had produced the metabolic disturbance.

Experimental pancreatic diabetes: Although clinical diabetes associated with gross lesions of the pancreas is a relatively rare picture even today, enough cases similar to that of Cawley were observed during the course of the next hundred years so that the relationship of the pancreas to diabetes was suspected by many investigators. In 1877 Lancereaux reviewed these cases, adding two from his own experience, and stated definitely his opinion that in at least some cases of diabetes the primary disease was in the pan-



FIG. 1. Frontispiece from Brunner's *Experimenta nova circa Pancreas* depicting the operation of pancreatectomy being carried out in dogs The operations are being performed by six ladies, and the importance of the experiment is signified by the presence of Aesculapius, Eros, and Zeus (or possibly, Prometheus) at the demonstration. For further discussion see Major, H H. *Ann Med. History*, *3* 91 (1941).

erted its anti-diabetic function through the activity of an internal secretion (24, 23). Conclusive proof of this concept was provided by the subsequent experiments of Minkowski and of Hédon who demonstrated that if the entire pancreas were removed except for a small subcutaneous graft, there was no metabolic impairment, but when the graft was excised, the typical diabetic picture appeared within a few hours (28, 16). This was almost incontestable evidence for the endocrine theory.

The islets of Langerhans: Diamare in 1889 and Laguesse in 1893, on the basis of histological and embryological studies, postulated that the endocrine tissue of the pancreas consisted in the nests of epithelial cells which had been described by Langerhans in 1869 (22, 19, 12) It was Laguesse who proposed the name "Les Îlots de Langerhans" for these structures. That the islets were the source of the internal secretion became accepted on the basis of the work of Ssobolew and others who injected the pancreatic duct system with paraffin (29). While this procedure effected very complete destruction of the acinar elements which became replaced by fibrous tissue, the islets were unimpaired and diabetic symptoms did not appear

The apparent importance of the islets of Langerhans as an endocrine system stimulated further anatomical research as a result of which it was shown by Bensley and Lane that various methods of staining delineated more than one type of islet cell (21, 7). The most numerous were found to be two granulated types referred to by Lane as A and B cells. The alpha cells, as they are now called, are the less numerous of these types and tend to be located peripherally in the islet. In addition to these two prominent forms there are "D" cells and a fourth non-granular "C" cell which was described by Bensley in the guinea pig.

There is adequate evidence that the beta cells are the

creas (20). Experimental evidence for this relationship was lacking until 1889 when von Mering and Minkowski in Strassburg succeeded in producing characteristic diabetes mellitus in the dog by total extirpation of the pancreas (27).

Although von Mering and Minkowski were the first to recognize the presence of diabetes in the depancreatized animal, the operation had been attempted by many earlier investigators. As early as 1682, Conrad Brunner, the discoverer of the duodenal glands which bear his name, had successfully removed the pancreas from dogs and described his experiments in a book entitled *Experimenta Nova circa Pancreas atque Diatribe de Lympha* (10). The cryptic frontispiece to this little book depicts the pancreatectomies being carried out (Fig. 1). Whether Brunner actually produced diabetes in any of his animals will, of course, never be known, but one is led to believe that he did by the following description of one of his dogs from which he had removed both spleen and pancreas.

> *It was especially to be seen that the animal made water very frequently, and that he was very thirsty, drinking largely of water in proportion to the discharge of urine.*

Unfortunately, the author was not stimulated to investigate these observations further since he knew Malpighi had described excessive thirst in animals in which he had only ligated the splenic vessels (15) Subsequent investigators before von Mering and Minkowski apparently failed to accomplish complete removal of the pancreas, and in 1856 Claude Bernard stated that it was impossible to perform total pancreatectomy in the dog (8) He did carry out the operation in birds and though the situation so produced was not consistent with prolonged life, the symptoms of diabetes were not seen (9).

The experiments of von Mering and Minkowski were repeated by Lépine who first proposed that the pancreas ex-

extracts from fetal calf pancreas were also effective in alleviating the symptoms of diabetes They used fetal pancreas because at this stage (prior to four months) the glands contained islets, but no acinar tissue (4). Later MacLeod prepared similarly active material from the principal islets of teleost fish, in which the islet tissue consists in a separate organ, distinct from the digestive portion of the pancreas (26). These experiments proved beyond any doubt that the anti-diabetic principle was being recovered from the islets of Langerhans

Since the original technique devised by Banting was not practical for the commercial preparation of insulin, other methods of extraction were tried. With the collaboration of Collip, the Toronto workers then found that acidified alcohol could be used to extract a very potent and non-toxic preparation (5). This became the basis for the modern large-scale manufacture of insulin

Crystalline insulin: In 1926 Abel and his co-workers succeeded in crystallizing insulin and demonstrated that the molecule was a protein (1). This was the first isolation in crystalline form of an animal protein with specific biological activity, and it laid the foundation for the extensive investigation of insulin chemistry which has subsequently been carried out

FURTHER DEVELOPMENTS IN EXPERIMENTAL DIABETES

Some other discoveries in the methods of producing and of modifying experimental diabetes have greatly augmented our knowledge of the diabetic state and of the endocrine control of carbohydrate metabolism. They deserve mention in this historical introduction.

Alloxan diabetes: In 1937 Jacobs found that the intravenous injection of alloxan (mesoxalyurea), a substance with the following formula.

source of insulin. Early experiments dealing with the exhaustive degeneration of the islets which follows subtotal pancreatectomy showed that it was the beta cells which became damaged in this process (21). More recent observations of the pancreas in pituitary and alloxan diabetes have yielded similar findings (Chapter V).

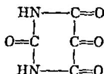
The discovery of insulin: During the thirty years which followed the experiments of von Mering and Minkowski innumerable attempts were made to prepare extracts of pancreas with anti-diabetic activity. There is little doubt that many of these earlier investigators did obtain extracts with hypoglycemic properties. The preparations were either too toxic or insufficiently potent or the methods for evaluating them were not adequately refined so that no convincing results were achieved before the work of Banting and Best in 1921 (3). Sir Frederick Banting had conceived the idea for his experiments after reading an article by Moses Barron concerned with the degenerative changes in the human pancreas which follow occlusion of the ducts by calculi (6). Banting and Best ligated the pancreatic ducts of dogs, allowed enough time for the acinar tissue to degenerate, and then removed the atrophied pancreases and extracted them with cold Ringer's solution. Extracts thus produced possessed striking ability to lower the blood sugar of depancreatized animals (3). The use of degenerated pancreas avoided the introduction of toxic pancreatic enzymes into the recipient animals and probably prevented some enzymatic destruction of the insulin This aspect of their procedure, however, was probably not alone responsible for the success of these workers where others had failed. The use of more accurate methods for blood sugar determinations and the more frequent withdrawal of blood samples for analysis contributed significantly to their success.

Following the preparation of insulin from the sclerosed pancreatic remnants, Banting and Best found that similar

of C. N. H. Long who showed adrenalectomy had a similar effect (25, 17). Since adrenal and pituitary hormones possess activity opposite to that of insulin on the blood sugar, their action is of obvious importance in the pathogenesis of the diabetic state

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produced in rabbits a transient period of hyperglycemia followed by severe and fatal hypoglycemia (18). Dunn, Sheehan, and McLetchie found that this phenomenon was associated with necrosis of the islets of Langerhans (14). They subsequently discovered that alloxan could be used to cause permanent diabetes in rats, and Bailey and Bailey succeeded in producing permanent diabetes in the rabbit by protecting the animals with glucose during the hypoglycemic period (13, 2). In most species the toxic action of this chemical is limited to the beta cells. The discovery of alloxan has lent tremendous impetus to the investigation of experimental diabetes by offering a technique for destroying the insulin-producing tissue alone without extirpation of the remainder of the pancreas, and by extending the investigation of experimental diabetes to species in which pancreatectomy is not feasible.

The discovery of other endocrine glands involved in the regulation of carbohydrate metabolism: There is not space in this monograph for a discussion of the role of endocrine mechanisms outside of the pancreas in the regulation of the blood sugar It must be emphasized, however, that the consideration of this regulation with reference to the pancreas alone is a very artificial approach since the momentary level of the blood sugar represents the equilibrium of a great many metabolic reactions which are catalyzed by secretions of the thyroid, the pancreas, the adrenals, the pituitary, and possibly other endocrines as well. Of importance in the development of the present concept was the discovery by Houssay that hypophysectomy would alleviate the symptoms of pancreatectomy diabetes and that

CHAPTER II

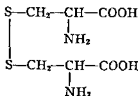
THE NATURE OF INSULIN

A comprehensive discussion of the chemical and physical properties of insulin is beyond the scope of this brief treatise, and excellent reviews on this subject are available (5, 6). A few general considerations in regard to the chemical nature of insulin, however, are pertinent to the discussion of the pancreatic hormone in subsequent chapters.

CHEMISTRY

Insulin is a protein with physical properties not differing greatly from other animal proteins of similar size. Though the molecular weight on the basis of ultracentrifuge determinations is now considered to be about 48,000, recent studies indicate that under the influence of change in pH or dilution, the molecule dissociates reversibly into four fragments with molecular weight of 12,000 (3). It is believed that these sub-molecules are normally held together by relatively weak polar forces Insulin is readily soluble in either acid or alkaline solutions, the isoelectric pH being 5 30-5.35.

The amino acid composition of the hormone is fairly characteristic for animal proteins, the only unusual feature being the very high proportion of sulfur in the molecule (3.2%) It was discovered by du Vigneaud that this sulfur exists entirely in the disulfide (S—S) form rather than the sulfhydryl (S—H) form and consequently must be accounted for by an unusually high proportion of the amino acid cystine (14):



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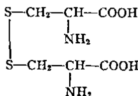
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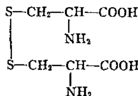
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The intactness of this disulfide linkage in cystine is one of the requisites for physiological activity of the hormone. The results of recent analyses of the amino acid composition of insulin appear in Table I.

TABLE I

AMINO ACID COMPOSITION OF INSULIN
(Grams per 100 grams protein)

Glycine	4.5
Valine	9.1
Leucine	13.0
Isoleucine	2.8
Cystine	11.7
Serine	6.6
Threonine	3.5
Arginine	3.4
Histidine	5.28
Lysine	2.4
Phenylalanine	7.95
Tyrosine	12.2
Glutamic acid	19.9
Proline	2.9

(From Velick, S. F. and Ronzoni, E. (13).)

IS INSULIN A SINGLE CHEMICAL SUBSTANCE?

Crystalline insulin satisfies the ordinary physical criteria for a single substance, since it is crystalline and under appropriate circumstances gives ultracentrifuge and electrophoretic patterns characteristic of a single component (11, 3, 4). However, none of these criteria are adequate to prove that the substance is truly a single protein. It is of interest, in this regard, that very impure insulin preparations containing as little as 16 international units per milligram give the electrophoretic pattern of a homogeneous substance as do highly purified crystalline preparations possessing 23 units of activity per milligram (4). Electrophoresis at pH 3.8, however,

has recently been shown to give two components, and, as will be seen later (Chapter VI), there is physiological evidence that even highly purified crystalline insulin is composed of more than one active substance (12).

The presence of zinc in the insulin molecule has incited considerable interest. It was shown by Fisher and Scott that the pancreas contains a large amount of zinc (about 20 milligrams per kilogram of tissue). Scott found that crystalline insulin contained 0.52% of zinc and that crystallization could be facilitated by the addition of zinc salts (2, 10). Amorphous insulin having been subjected to electrodialysis and retaining as low zinc content as .04% manifests full activity, however, and crystalline insulin with 0.15% zinc content has been prepared. It appears therefore that, although this element is regularly found in insulin preparations, its presence is not necessary for physiological activity.

CHEMICAL MODIFICATIONS OF INSULIN

Almost any chemical change which can be carried out on the insulin molecule results in serious or complete loss of pharmacologic potency. Acid or enzymatic hydrolysis of the protein into its constituent amino acids results in total loss of activity as does treatment with alkali (9). Allowing the material to stand in acid alcohol also causes loss of activity which is partially reversible by treatment with alkali (1). Acetylation of the free amino groups by reaction with ketene or acetic anhydride also destroys activity indicating that these groups play an important part in the physiological properties of the substance (8, 7). Of particular interest is the inactivation which results from reduction of the cystine disulfide linkage by weak reducing agents like cysteine and glutathione (15, 16) In this case there is evidence that the inactivation may be related not only to the chemical

modification at this point but to molecular rearrangements which follow disruption of the (S—S) bond. As will be seen later (Chapter VI), the reaction has been used to separate the functions of two active entities in commercial insulin preparations.

CHEMICAL CHARACTERISTICS OF INSULIN RESPONSIBLE FOR ITS ACTIVITY

Following the demonstration that insulin was a protein, it was widely suspected that the substance must exert its physiological action through some prosthetic group or relatively small chemical grouping which was either adsorbed on or included within the molecule. It was an unfamiliar concept that an entire protein could possess such specific pharmacodynamic properties as those of insulin. Exhaustive study of the chemical constitution of the purified hormone has failed to disclose the presence of any such grouping, and we must assume that the entire insulin molecule with its normal molecular and spatial configurations is necessary for physiological activity

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CHAPTER III

METABOLISM IN DIABETES AND THE ACTION OF INSULIN

THE situation which von Mering and Minkowski produced by total pancreatectomy in the dog qualitatively duplicated the entire picture of spontaneous human diabetes mellitus (38). Polydypsia, polyphagia, hyperglycemia, glycosuria, and ketosis, which rapidly became evident after the operation, were followed by acidosis and dehydration and progressive cachexia leading to the animal's death The choice of the dog as an experimental animal was a fortunate one, for, as will be seen later (Chapter VI), not all animals respond to pancreatectomy with this typical syndrome. The constant association of all these phenomena in both pancreatectomized dogs and human diabetes suggested that they were a related series of events resulting from a single metabolic defect. The subsequent investigations of Minkowski and his school and those of Lusk and many other workers in this country produced a simple unified theory as to the metabolic impairment in diabetes and the pathogenesis of the diabetic syndrome (39, 36). The development of this original theory and the modifications of it which were made necessary by subsequent investigations are discussed below

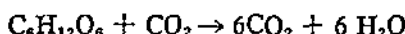
OVER-ALL METABOLISM IN EXPERIMENTAL DIABETES

Early studies on pancreatectomy diabetes in the dog revealed, in addition to hyperglycemia, glycosuria, and ketosis, low levels of liver glycogen and characteristic findings with regard to the respiratory quotient (R Q) and the dextrose to nitrogen (D:N) ratio (39)

Respiratory quotient: The R Q. which represents the fraction

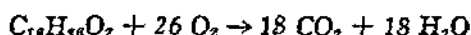
$$\frac{CO_2 \text{ produced}}{O_2 \text{ consumed}}$$

when determined in the resting animal in the post-absorptive state was considered to be an index of the type of foodstuff being metabolized. Thus if the over-all metabolism of glucose is represented by the formula:



$$C_6H_{12}O_6 + CO_2 \rightarrow 6CO_2 + 6\ H_2O$$

the R.Q. of such a process is 1 00 since equimolar amounts of oxygen and carbon dioxide are involved. The oxidation of an average fatty acid, on the other hand, which might be represented as follows:



$$C_{18}H_{36}O_2 + 26\ O_2 \rightarrow 18\ CO_2 + 18\ H_2O$$

would have an R Q. of 18/26 or approximately 0.7.

The R.Q. calculation for the animal as a whole can be modified to eliminate the contribution of protein metabolism by measurements of the nitrogen excretion and the assumption that each gram of urinary nitrogen represents 6 25 grams of protein metabolized. This corrected value or non-protein respiratory quotient (NPRQ) was felt to represent the proportion of fat and carbohydrate undergoing oxidative degradation (36).

The respiratory quotients of depancreatized dogs fall immediately to values around 0.71 after operation and the administration of glucose was found not to cause the increase in R Q. which follows glucose administration in the normal animal (45, 2) On this basis, the conclusion that the totally diabetic animal could not metabolize carbohydrate at all was reasonable.

The D:N ratio: The other quantity which received a great deal of discussion in the early work in experimental diabetes

was the D:N ratio. Minkowski and subsequent workers had shown that in the fasting pancreatectomized dog which had been fed on a meat diet, the ratio of glucose to nitrogen in the urine was a very constant value between 2.8 and 3.0 *(39, 13)*. This was construed to mean that when protein was the only source of carbohydrate, the D:N ratio represented the maximum amount of glucose that was obtainable from protein and that this amount was excreted in the urine. When to such an animal carbohydrate was given, the latter could be quantitatively accounted for in the subsequent increase in glycosuria and this was in harmony with the concept that no carbohydrate at all could be catabolized in the diabetic. It was not explained, however, why the D:N ratio for the "totally" diabetic animal was lower than that reported by Lusk (3.65) for the phlorizinized animal *(36)* in which the ability of the kidney to retain glucose is the primary defect.

Failure of combustion of fats: With the theory that the basic anomaly in diabetes was the inability to utilize carbohydrate, the phenomenon of ketosis remained to be explained. The prevailing opinion was that the complete combustion of fats required the *simultaneous* combustion of carbohydrate. In the absence of carbohydrate utilization fats accumulated at the "half-burned" stage—namely ketone bodies. This principle gave rise to the well known aphorism that "fats burn in the flame of carbohydrate" and eventually to precise stoichiometric formulations of the coupling of carbohydrate with ketone bodies in their final oxidation *(49)*.

WEAKNESSES OF THE ORIGINAL THEORY OF DIABETIC METABOLISM

With the development of greater understanding of the details of metabolic mechanisms, the original scheme became

somewhat weakened The evidence for the primary defect in carbohydrate metabolism is now less clear-cut, whereas the original postulate concerning the derangement of fat metabolism is untenable.

Criticism of the respiratory quotient: In recent years the validity of the respiratory quotient as an index of the type of foodstuff undergoing oxidation has been challenged (56). As knowledge of the processes of intermediary metabolism has increased, it has been apparent that the three major elements of metabolism are not merely burned by their own separate pathways, but are constantly being converted one to another for purposes of both storage and degradation. Such interconversions obviously influence the R.Q in one direction or the other, depending upon whether molecules of higher or lower oxygen content are being produced. An example of such an interconversion was contained in the demonstration by Stettin and Boxer that 30% of glucose ingested by the normal fasted rat was metabolized by way of conversion to depot fat (62). It is apparent then that the normal R Q. represents the net result of a great many reactions. A decrease in R Q. does not necessarily indicate decreased oxidation of carbohydrate, but theoretically could have many other explanations—such as conversion of fat to carbohydrate or failure of conversion of carbohydrate to fat

Interpretation of the D:N ratio: The significance of the D·N ratio as an index of the basal excretion of carbohydrate derived from protein is open to question. Soskin has reported a much wider range of values than those recorded by Minkowski (56) The value is apparently not a constant one and it is doubtful whether the classical interpretation of this ratio has any firm justification

Ketone utilization: It is now certain that the accumulation of ketone bodies in the diabetic organism is not a result of

a defect in the ability to metabolize them. This was shown by Chaikoff and Soskin (12) and amply confirmed by Stadie who demonstrated that diabetic cats are able to destroy administered acetoacetate as readily as normal animals Muscle mince from diabetic animals possessed similar ability (60, 57). Moreover, insulin, in Stadie's experiments, was able to inhibit ketogenesis in isolated liver slices. It appears that the liver is alone capable of producing ketone bodies from fatty acids. Since they are metabolized in the peripheral tissues of the diabetic as well as in normal tissues, it follows that they must be produced in excess in the diabetic.

It is now clear from the work of Mirsky and others that the critical factor concerned with ketogenesis is the level of *liver glycogen (42, 40). Depletion of liver glycogen,* regardless of cause, stimulates ketogenesis from fatty acids. The mechanism of this stimulation is not known, but it occurs in starvation, phlorizin poisoning, and even in hypoglycemia when, as Somogyi has shown, the glycogen stores are depleted by excessive doses of insulin (53). It was also found that even in uncontrolled experimental diabetes, the injection of massive amounts of glucose would reverse ketogenesis if quantities sufficient to cause hepatic glycogen formation were given (41).

Recently Yoshikazu and Orten demonstrated that in alloxan-diabetic rats, which for some reason are able to maintain relatively high fasting glycogen stores, severe ketosis does not occur (65).

DIRECT EVIDENCE CONCERNING THE IMPAIRMENT OF CARBOHYDRATE METABOLISM AND THE EFFECT OF INSULIN

The weight of evidence now available indicates that, while utilization of carbohydrate does occur in the diabetic, its rate is quantitatively reduced. The effect of insulin is to

accelerate this process. Various types of more or less direct experiments have supplied the evidence for the failure of utilization, and some of these are quoted below.

Evidence for effect of insulin on peripheral (extrahepatic) utilization of carbohydrate: As early as 1912, Knowlton and Starling reported that glucose was removed from the perfusing blood of canine heart-lung preparations less rapidly when the preparations were taken from diabetic animals (28). Though these experiments were done in the pre-insulin era, they constituted good evidence that the antidiabetic factor influenced the uptake of glucose by peripheral tissues. Similar evidence of a direct nature was obtained in balance experiments such as those of Best, Hoet, and Marks who, using eviscerated cats, showed that insulin accelerated the disappearance of glucose and the concomitant uptake of oxygen (4). Similarly, Mann, using hepatectomized animals, found that though the blood sugar fell rapidly in diabetic animals following hepatectomy, the drop could be accelerated by the addition of insulin (37). Thus insulin was able to act on peripheral tissues in the absence of the liver. The hepatectomy experiments of Mann showed, however, that the peripheral utilization of glucose proceeds rapidly in the diabetic and that the action of insulin on this process is only a quantitative one. Less direct evidence for the peripheral action of insulin on glucose utilization is to be found in the numerous experiments dealing with arteriovenous glucose differences It is generally agreed that the peripheral A-V glucose difference is decreased in the diabetic organism and can be caused to increase by the administration of insulin (17).

Evidence for the effect of insulin on the metabolism of completely isolated tissues is less convincing An example of such an *in vitro* demonstration of the action of insulin which has ben substantiated was that of Krebs and Eggle-

ston who found that the pancreatic hormone increased the oxygen consumption of pigeon breast muscle (29). While this observation was confirmed by Stadie and by Shorr, no comparable effects were observed in mammalian muscle preparations (51, 59). Stare and his colleagues, on the other hand, did observe effects in mammalian muscle and found that insulin increased oxygen consumption in muscle preparations taken from insulin-sensitive diabetic patients. It was ineffective, however, in preparations from normal individuals or insulin-resistant diabetics (61, 47). Although numerous other claims for direct *in vitro* effects of insulin have been made, little confirmation for them exists. The indirect effect of insulin on the *in vitro* phosphorylation of glucose is described below.

Glycogen formation: Experiments concerned with the action of insulin on glycogen formation have introduced an interesting paradox. There is no question that in the diabetic organism, glycogen, at least in the liver, becomes severely depleted and that if carbohydrate is available, insulin is able to restore the glycogen reservoirs (1). Most investigators agree, however, that the usual action of insulin in the normal organism is to release glycogen from the liver while stimulating glycogen formation in muscle (6, 5, 11, 63, 20). Shipley and Hümel, on the basis of studies relating to the effect of insulin on liver slices, concluded that insulin causes hepatic glycogenolysis if the existing glycogen level is high and glycogenesis if the initial level is low (50)

The formation of glycogen in muscle under the influence of insulin was demonstrated in isolated rat diaphragm by Gemmil and has recently been confirmed by Bartlet and coworkers utilizing the radioactive C_{14} technique (23, 3). As will be discussed later (Chapter VI), the conflicting findings regarding liver glycogen formation may be resolved by the finding of Sutherland and Cori that insulin preparations

contain two factors with opposing activity in regard to hepatic glycogenesis.

THE "OVER-PRODUCTION THEORY"

The paucity of direct evidence for the failure of carbohydrate utilization and the weaknesses of the original theory discussed above have led to the development of an alternative hypothesis which has some adherents. This hypothesis, originally suggested by von Noorden, is supported at present mainly by Soskin and his associates (43, 54, 56).

No defect in carbohydrate utilization: It is the conviction of this school that no defect exists in the peripheral metabolism in the diabetic but rather that hyperglycemia is initiated by an overproduction of glucose by the liver. Key experiments in relationship to this theory are those of Soskin concerning glucose utilization in hepatectomized and eviscerated dogs with and without the presence of the pancreas (55). It is Soskin's finding that at the blood sugar levels which exist in these two preparations, glucose disappearance proceeds at the same rate in eviscerated animals with and without pancreas. The initial blood sugar levels were naturally higher in the pancreatectomized animals. When, on the other hand, the rates of disappearance are considered at equivalent blood sugar levels, the diabetic animal manifests a slower rate of glucose removal. From these experiments, Soskin has described the glucose metabolism of the depancreatized animal as "qualitatively normal" However, one cannot feel that the ability of the diabetic tissues to metabolize carbohydrate is truly normal, since they require higher than normal blood glucose levels to maintain the normal rate of carbohydrate uptake

Proponents of this over-production theory have also cited the well known fact that certain tissues such as brain can metabolize only carbohydrate (25), and they point out that

there is no evidence that the function of the central nervous system is preferentially compromised in the diabetic.

Carbohydrate from fat: If, as the proponents of the "over-production theory" contend, the utilization of carbohydrate proceeds at a normal rate in the diabetic, the problem arises of the sources of the carbohydrate which is metabolized plus that which is excreted in the urine. Since the amount of nitrogen excreted does not indicate that the excess carbohydrate can all come from protein, it is necessary to postulate that a large portion of this material comes from fat. The possibility of this conversion has been the basis of a heated argument for many years and has been reviewed extensively in Soskin's works (56, 54). The work of Buchanan, Hastings, and Nesbitt showing that radioactive carbon (C_{11}) fed as carboxyl-labelled butyric and propionic acids could be recovered in liver glycogen seems to indicate at least that pathways for this transformation exist (9). The question of to what extent such a process actually occurs in the diabetic remains to be elucidated. There is at present no evidence that a *net* synthesis of carbohydrate from fat ever takes place.

It has been repeatedly questioned whether the diabetic liver actually produces an excess of glucose as the partisans of over-production claim This has not been proven and in experiments of Crandall and Lipscomb in which the hepatic glucose output was calculated from blood flow determinations and sugar concentrations, there was found to be no significant increase in hepatic glucose output in the diabetic animal (18).

ATTEMPTS TO FIND A PLACE FOR INSULIN IN THE ENZYMATIC MACHINERY OF METABOLISM

Modern enzyme chemistry has learned a great deal in recent years concerning the steps by which carbohydrate is

degraded and the enzyme systems which enable these transformations to proceed. One might expect that insulin would be found to act on one of these steps or possibly on one enzyme system which might be concerned with several steps. Although some interesting findings have been made with regard to the effect of insulin in these systems, neither of these expected types of action has been finally proven.

Scheme of carbohydrate breakdown: Figure 1 is an abridged representation of what many feel to be the important steps in carbohydrate breakdown. Glucose entering the body is transformed to glycogen through the intermediate steps of phosphorylation The subsequent breakdown of glycogen to yield energy can be considered in two main stages. In the first stage or "glycolytic cycle," glycogen is converted to the three-carbon substance, pyruvate, or in the absence of oxygen, to lactate. The second or "oxidative cycle" is initiated by the condensation of pyruvic acid with oxaloacetic to form isocitric acid. Through this "tricarboxylic acid cycle" of Krebs three molecules of CO_2 and two molecules of water are given off and oxaloacetic acid is regenerated for further condensation with pyruvate In the glycolytic stage there is only one oxidative step: the formation of 1:3 diphosphoglyceric acid from 1:3 diphosphoglyceraldehyde This oxidation consists in the removal from the latter substance of hydrogen, which becomes associated with the coenzyme diphosphopyridine nucleotide (DPN) The hydrogen, in the presence of oxygen, is passed on through flavin to the cytochrome system through which it is eventually oxidized to water. In the absence of oxygen the hydrogen from reduced DPN is used to reduce pyruvic acid to lactic acid. The remainder of the energy-producing oxidative steps occur in the tricarboxylic acid cycle

The energy from these oxidative reactions is not released in the form of heat, but used to form substances possessing

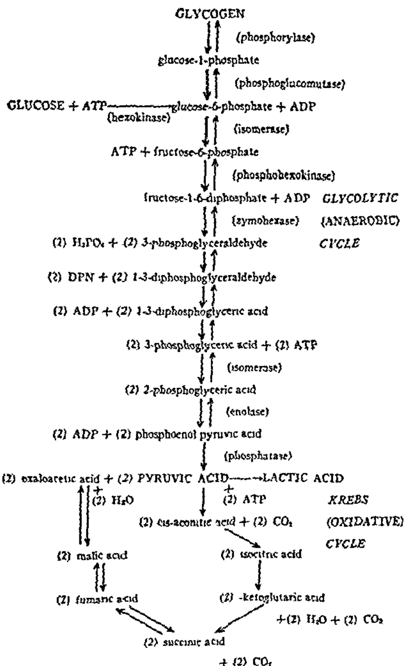


FIG 1 The major steps and the enzymes involved in the oxidation of glycogen to carbon dioxide and water

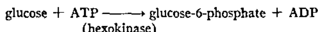
very high energy which can be subsequently liberated on their breakdown. This energy is in the form of phosphate bonds, and the important known "high energy phosphate bond substances" are adenosinetriphosphate (ATP) and creatine phosphate. The former substance is probably the immediate source for mechanical energy such as is required for muscle contraction The latter is perhaps only a "storage" form of phosphate bond energy

Stage of carbohydrate degradation upon which insulin is effective: "pre-pyruvate" or "post-pyruvate"? The question of which stage of carbohydrate oxidation is interrupted in the diabetic is not settled. In the original experiments of Krebs in which it was shown that insulin increased respiration in pigeon muscle mince, it was found that this effect of insulin was enhanced by alpha-ketoglutarate, succinate, fumarate, and oxaloacetate—i.e , the components of the "Krebs cycle" (29). It was felt from this that the site of action insulin was on the "oxidative cycle" of carbohydrate metabolism. Stadie, Zapp, and Lukens, on the other hand, duplicating the experiments of Krebs, found that citrate had no effect on this action of insulin (59)

Recent evidence suggesting that insulin acted on the metabolism of pyruvate was contained in the experiments of Charalampous and Hegsted (14) They found that the acetylation of para-amino benzoic acid was retarded in the alloxan diabetic rat. This function could be restored by the addition of members of the tricarboxylic acid cycle. It could not be influenced, however, by the addition of pyruvate. The work of Flock, Bollman, and Mann, however, militates against the idea that insulin affects the oxidation of pyruvate. They demonstrated that administered pyruvate could be utilized at a normal rate in the depancreatized dog. This utilization was accompanied by an increase in lactate such as is seen during pyruvate metabolism in normal animals (22).

The evidence as to whether insulin may act before pyruvate, i.e., on the glycolytic cycle of carbohydrate metabolism, is also difficult to interpret. The resting levels of pyruvate and lactate in the diabetic organism are not characteristically abnormal. It has been shown, however, that the rise in pyruvate and lactate which follow the administration of glucose in the normal animal does not occur in the diabetic, whereas an increase in both lactate and pyruvate can be produced in the diabetic by the simultaneous administration of glucose and insulin (27, 10). The latter experiments seem to constitute good evidence for the action of insulin somewhere prior to pyruvate.

The hexokinase reaction: The most specific action which has been ascribed to insulin is contained in the work of Cori and his co-workers relevant to the hexokinase reaction. The first step in the utilization of glucose, either for degradation or for glycogen synthesis, is the formation of glucose-6-phosphate. The enzyme for this reaction is hexokinase and the phosphate is contributed by adenosine triphosphate. This reaction which can be carried out *in vitro* is as follows:



Price, Cori, and Colowick showed that tissues from alloxan-diabetic rats or rats which had received anterior pituitary injections possessed subnormal hexokinase activity Anterior pituitary extracts when added to the constituents of this reaction *in vitro* inhibited its progress Insulin was found to have no effect on the reaction per se but was able to abolish the inhibitory effect of anterior pituitary extract (44) It was shown further that adrenal extract could depress the hexokinase activity of muscle preparations taken from alloxan-diabetic rats, though it could not influence preparations

from normal animals. As with anterior pituitary extract, the inhibitory effect of adrenal cortical hormone could be released by the addition of insulin (16).

The fact that both the anterior pituitary and the adrenal cortex act to oppose insulin with respect to the blood sugar is well known, and the findings of Price, Cori, and Colowick help to explain the Houssay animal and Long's doubly-operated cats (26, 19, 32). They do not, as yet, explain the entire action of insulin, since it is well recognized that insulin does not require the presence of the pituitary for its action. The hypophysectomized animal is indeed extremely sensitive to the hypoglycemic action of insulin The situation is further complicated by the fact that several recent investigators have not been able to confirm the work of Price, Cori, and Colowick (45, 52, 15, 58, 8). Nevertheless, the idea that insulin acts on the initial phosphorylation of glucose is a highly satisfactory one. There is no good evidence that insulin influences any single step in either the anaerobic or the oxidative cycle of metabolism and there is in diabetes no "piling up" of any substance in the metabolic chain save glucose itself. Thus an action of insulin on the hexokinase reaction would be consistent with the main body of experimental facts and would explain both the apparent deficiency of utilization and the failure of glycogen formation in the diabetic

Effect of insulin on diffusion and membrane permeability: The insufficiency of evidence to demonstrate that insulin is effective in isolated cell-free systems has led some to the conclusion that insulin may act by increasing the rate of entry of glucose into the cells where it is metabolized. Some experimental justification for this belief exists in the recent work of Levine and his co-workers who found that insulin increased the dispersion of galactose in eviscerated nephrectomized dogs (30) Since galactose is not metabolized without first conversion to glycogen in the liver, the authors

concluded that insulin aided in the passage of hexose through cell membranes.

MISCELLANEOUS ACTIONS OF INSULIN ON *SUBSTANCES OTHER THAN CARBOHYDRATE INTERMEDIARIES*

Plasma amino acids: It was originally shown by Luck that the concentration of free amino acids in the blood falls rapidly following the administration of insulin. This seems to indicate that the action of insulin in preventing the severely negative nitrogen balance of uncontrolled diabetes involves inhibiting the breakdown of proteins to amino acid rather than in preventing the deamination of the latter (35, 34). That these substances form protein under the influence of insulin is suggested in the work of Lotspeich who showed that the rates of disappearance of the separate amino acids following insulin injection was proportional to their individual concentrations in structural tissues (33).

Inorganic phosphate: Following the injection of insulin, there occurs a rapid fall in the blood inorganic phosphate level (64). Administration of glucose results in a similar fall in the normal, but not in the depancreatized animal. The question arises as to whether this represents a fundamental action of insulin on phosphate metabolism. That such may be the case was suggested by the finding of Sachs that insulin increased the rate of turnover of radioactive phosphorous in ATP and by the findings regarding the hexokinase reaction quoted above (48, 44).

Levine and his co-workers have recently shown, however, that the administration of fructose or very large amounts of glucose result in a lowering of the serum phosphate levels in depancreatized dogs (31). This they feel is evidence that the fall in inorganic phosphate level is merely the consequence of the rapid entry of hexose into

the tissues and does not represent a direct action of insulin.

Serum potassium: As with inorganic phosphate, a fall in serum potassium levels is regularly seen following the injection of insulin (7, 24). The entire significance of this is not known, but as Fenn showed, the deposition of glycogen in the liver is accompanied by the deposition of potassium (21). This apparently does not account for all of the potassium which is removed from the blood under the influence of insulin. The phenomenon has important clinical significance, since in the insulin treatment of diabetic coma severe hypokalemia may result after the patient's metabolic status has been returned to normal in other respects.

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CHAPTER IV

THE REGULATION OF INTERNAL PANCREATIC SECRETION

AN INTRIGUING question and one of practical consequence is concerned with the factors which regulate the release of insulin into the blood stream. This question might be stated more broadly: Does the pancreas release variable amounts of internal secretion depending on the need for it, *and if so, what mechanisms regulate this process? The manner* in which insulin is produced to regulate carbohydrate metabolism is worthy of study since clinical experience seems to indicate that the most effective regime for control of the diabetic with insulin must be an imitation of the manner in which insulin is secreted in the normal organism.

THE CHARACTERISTIC CHANGES IN BLOOD SUGAR FOLLOWING CARBOHYDRATE ADMINISTRATION

The glucose tolerance curve: There are aspects of the normal glucose tolerance curve which indicate that, following the elevation of the blood sugar by exogenous glucose, the blood level does not merely drop at the basal rate of utilization, but rather that the mechanisms for removing the substance from the blood are accelerated. In the normal individual, for example, following induced hyperglycemia, the curve of glucose concentration usually drops for a short period below the initial, fasting level. This indicates that the acceleration of glucose removal persists, for a short time, after the blood sugar has been returned to normal (Fig 1).

Furthermore, if after the blood sugar has started to fall, another dose of glucose is given, little or no increase in the blood sugar will be observed as a result of the second administration (40, 41, 42). If, as has been repeatedly demon-

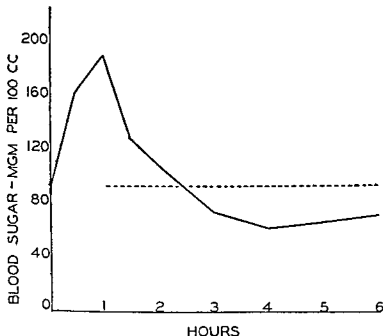


FIG 1 Five-hour glucose tolerance curve in normal individual, illustrating "hypoglycemic phase" of blood sugar following glucose administration (From the laboratories of the University of Minnesota Hospitals)

strated, continuous infusion of glucose is maintained, the blood level rises immediately and then falls, duplicating the glucose tolerance curve (6, 10, 43, 7) If then the infusion is continued, the levels may remain low for several days depending on the rate of infusion. A rapid secondary rise occurs at the time when the animal's reservoirs for carbo-

hydrate storage have been entirely filled. This type of experiment is illustrated in Figure 2 with data taken from the work of Barron and State (7). Such an experiment illustrates the fact that the mechanisms for removal of large amounts of glucose from the blood stream must be activated

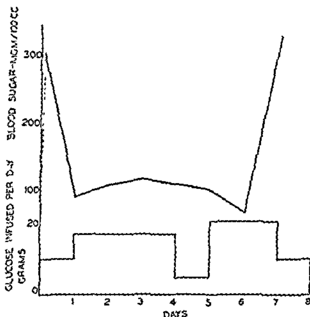


FIG. 2. Blood sugar of normal dog during continuous intravenous infusion of glucose. Illustrates return to normal after initial hyperglycemia and secondary rise on sixth day when glycogen stores have become completely saturated. (Graph constructed from data of Barron and State (7).)

by the presence of excess quantities of glucose. The lag-period during which this activation occurs is reflected in the hyperglycemic portion of the glucose tolerance curve. Once these mechanisms are put into action, extremely large amounts of glucose can be given without perceptible hyperglycemia.

Effect of previous diet: Related to this phenomenon are the familiar facts with regard to the effect of diet on carbohydrate tolerance (40, 23). High carbohydrate intake increases carbohydrate tolerance, whereas limitation of food, particularly carbohydrate, decreases it ("starvation diabetes").

Early workers felt that the acceleration of glucose utilization consequent to the administration of carbohydrate was the result of direct stimulation of enzymes in the liver (40), but after the conclusive demonstration of the existence of the pancreatic hormone, experiments were designed to decide whether the variations in glucose tolerance could be the result of changes in the rate of insulin production. Some of these experiments which are cited below were devised by very ingenious investigators.

THE INTRINSIC REGULATION OF INSULIN PRODUCTION

In 1927, Zunz and La Barre reported the results of cross-circulation experiments in which the pancreatico-duodenal vein of one dog was connected to the jugular of a second (45). Figures 3 and 4, reproduced from their work, illustrate the experimental preparation and the results. It was their finding that when the blood sugar of the donor animal was elevated by the injection of glucose, the blood glucose concentration fell in the recipient animal. This was interpreted to be the result of additional insulin secretion in response to the stimulus of hyperglycemia.

In the same year Gayet and Guillaume reported the results of transplanting a pancreas to the neck of a dog by connecting its vessels to the carotid artery and jugular vein (18, 19) They found that this procedure did not influence the blood sugar of a normal recipient, but would lower the hyperglycemia of a depancreatized recipient dog. When

glucose was introduced into the vessel leading to the transplanted pancreas, a fall in blood sugar ensued. The same result was obtained by Foglia and Fernandez using a similar preparation (17). Analogous results were reported by Kosaka who studied the effect on peripheral blood sugar of

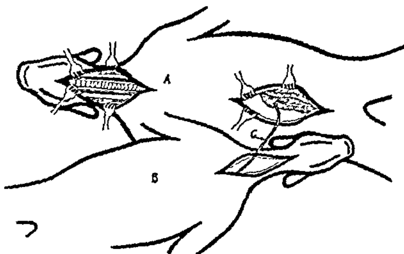


Fig 3. Illustration from work of Zunz and La Barre (45) showing experimental setup for studying release of insulin from the pancreas The pancreatic vein of the donor dog A is connected to the jugular vein of recipient dog B

glucose introduced into the pancreatico-duodenal artery and by London and Kotschneff who determined the insulin content of blood from the pancreatico-duodenal vein following the artificial elevation of the animal's blood sugar (27, 29). The latter investigators noted incidentally that the increase in insulin output persisted after the glycemia had returned to normal.

In more recent years, the intrinsic ability of the pancreas to respond to hyperglycemia has been admirably demonstrated by the work of Anderson and Long (1). They studied

the insulin output of an isolated pancreas in a specially-designed perfusion pump (30). The insulin content of perfusate coming from the isolated organ was determined by bioassay utilizing the extremely insulin-sensitive hypophysectomized-adrenal-demedullated-alloxan-diabetic rat (3). An increase in the output of insulin by the isolated pancreas was readily demonstrated by this method when the glucose concentration of the perfusing fluid was elevated. That the pancreas can by itself respond to hyperglycemia with increased insulin output seems therefore to be established beyond question.

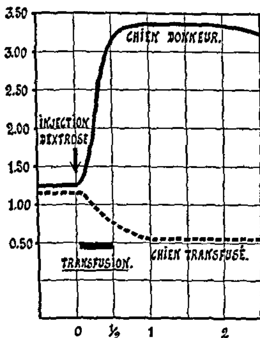


FIG 4 Graph from work of Zunz and La Barre (45) showing hypoglycemia in recipient dog following injection of glucose in donor dog to which former is connected by pancreato-jugular anastomosis.

THE ROLE OF NERVOUS CONTROL IN THE REGULATION OF INSULIN SECRETION

Although the function of the vagus nerve in the regulation of insulin secretion had previously been suspected (15, 13), Britton reported the first convincing experiments in which peripheral stimulation of the vagus was found to produce a decrease in blood sugar (9). This finding has been amply confirmed. Clark, in the same year, found that certain drugs which were known to stimulate the parasympathetic system caused hypoglycemia and that this effect was eliminated by section of the vagi (12). Recently the work of McQuarrie and of Gellhorn and his co-workers has shown that generalized stimuli such as hypoxia, emotion, and metrazol which ordinarily produce hyperglycemia will cause hypoglycemia when the activity of the adrenal medulla is removed (32, 16, 21, 20). This effect is abolished by section of the vagi. It represents an antagonistic function on the part of the "vago-insulin system" which apparently modifies the hyperglycemic activity of the "sympatho-adrenal system" under this type of stimulation.

That the parasympathetics can stimulate insulin secretion is beyond doubt The question remains to what extent they are involved in the normal regulation of the blood sugar.

In the cross-circulation experiments of Zunz and La Barre mentioned above, it was found that the blood sugar fall in the recipient dog, consequent to hyperglycemia in the donor dog, was decreased if the vagi were cut or atropine was given (45). Further experiments were done with a three-dog preparation from which they concluded that there were receptors in the central nervous system which were responsive to the blood sugar and regulated impulses for islet stimulation going by way of the vagus. The location of these glucose-sensitive centers was later investigated by La Barre (28).

Although the role of the nervous system in the regulation

of insulin secretion and the blood sugar is not entirely clear, it is apparent that the islets can function normally without nervous connections. Houssay found only slight variations from the normal glucose tolerance curve in animals with denervated pancreases and in animals in which the entire function was being carried by a pancreatic graft (25, 26). Phillips reported entirely normal glucose tolerance curves in vagotomized animals (34).

It seems reasonable to consider the nervous control of insulin secretion as an accessory or emergency system whose primary function is perhaps to modify the hyperglycemic effect of the sympathetics.

THE QUESTION OF PITUITARY CONTROL OF PANCREATIC SECRETION

The concept of a "pancreatropic hormone" originated with Anselmino and Hoffman who demonstrated that an extract of the anterior lobe could cause hypoglycemia in the intact animal but not in the animal in which the pancreas had been removed (4, 5). Support to this idea was offered by the finding that some pituitary extracts can cause hypertrophy of the islets of Langerhans (11, 35, 33) and increase in the insulin content of the gland (31).

Many investigators on the other hand have failed to obtain extracts with the properties described by Anselmino and Hoffman (14, 36, 44). That the normal function of the pancreas depends on trophic activity of the pituitary seems unlikely since hypophysectomy produces no changes in secretory capacity of the islets (24). Haist has also shown that the insulin content of the pancreas is not altered by hypophysectomy when the controls are given the same diet as hypophysectomized animals will eat (22).

The recent demonstration by Anderson and Long that growth hormone inhibits the insulin output of the isolated

perfused pancreas indicated that at least one pituitary fraction exhibits the opposite of a "trophic" effect (2).

REGULATION OF THE BLOOD SUGAR IN THE ABSENCE OF THE PANCREAS

Although it has been established beyond any doubt that the pancreas does respond to increased demands by the release of excess insulin, it is also true that acceleration of the rate of carbohydrate dissimulation is not entirely dependent on changes in insulin concentration.

The decreased glucose tolerance associated with carbohydrate starvation has been demonstrated in the eviscerated animal indicating that changes in pancreatic secretion are not necessary for this adaptation to occur (8). In the experiments of Soskin and co-workers, blood sugar was maintained within normal limits in depancreatized animals by the continuous infusion of a "balanced" mixture of glucose and insulin. When an added amount of glucose was administered to such a preparation, the entire normal glucose tolerance curve including the hypoglycemic phase was reproduced despite the fact that no additional insulin had been introduced (38, 39, 37)

We must conclude then that the tissues which handle carbohydrate utilization and storage possess an intrinsic ability for regulating their rate of glucose uptake in addition to that which is conferred on them by the varying amount of insulin made available by the pancreas

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CHAPTER V

SPECIAL PROBLEMS IN LIPID METABOLISM

IN THIS chapter are briefly considered three aspects of lipid transport and metabolism which are related to pancreatic function. It is appropriate to mention these topics here because, although their status as examples of endocrine functions of the pancreas is neither proven nor widely accepted, they are questions which must be settled before our understanding of pancreatic endocrine physiology becomes complete.

THE LIPOTROPIC FACTOR

Following the discovery of insulin it was disappointing to find that pancreatectomized animals could not be maintained in good health indefinitely by the administration of the pancreatic hormone alone. This was reported by Fisher who observed that pancreatectomized dogs so treated developed extensive fatty metamorphosis of the liver to which they succumbed after a period of six to eight months (16). The finding was confirmed by Allen, Bowie, MacLeod, and Robinson who demonstrated that the inclusion of 100-200 grams of raw pancreas in the diet of such animals would prevent fatty liver and greatly prolong their lives (1). They believed the fatty liver to have been caused by a deficiency of the external pancreatic secretion. In 1930, Hershey discovered that the phospholipid lecithin could be substituted satisfactorily for raw pancreas in the prevention of fatty liver in depancreatized dogs (20). Best and his co-workers then reported that choline, which represents a portion of the

lecithin molecule, was as effective as lecithin in this respect (4). The discovery of this property of choline led to the investigation of a whole field of so-called lipotropic substances which are able to decrease the lipid content of fatty livers of various etiologies and whose deficiency will itself cause fatty change in the liver. This very important outgrowth of the early work on insulin has been reviewed by Best and cannot be discussed in detail here (3).

In 1936, however, Dragstedt and his co-workers introduced a different concept (29). They pointed out that the amounts of choline and lecithin required to maintain health in the pancreatectomized dog were far in excess of the quantities of these materials in the amount of pancreas required to produce the same effect. In addition, tissues such as liver and brain, which contain a higher phospholipid concentration than pancreas, did not possess the latter's activity on the fatty livers of insulin-treated pancreatectomized dogs. It was Dragstedt's finding that ligation of the pancreatic ducts alone did not eventuate in fatty liver nor would the administration of pancreatic juice to depancreatized animals prevent the occurrence of such changes (18). On the basis of this evidence he proposed that the pancreas produces a second internal secretion which acts on lipid metabolism in such a way as to prevent the accumulation of excess liver lipids. He succeeded in preparing an alcoholic extract possessing the characteristic activity of the whole gland in a daily oral dose of 60-100 milligrams (12, 8). Dragstedt named this material "lipocaic," which means "I bear fat."

Few people have accepted the idea that the lipotropic factor of the pancreas is an internal secretion. It is conceded, and more recent work has confirmed the fact, that the extracts possess greater lipotropic potency than can be accounted for by their content of known lipotropic agents

(32, 22, 13). Ralli and co-workers, contrary to the findings of Dragstedt, found that ligation of the pancreatic ducts did produce fatty changes in the liver (31). Chaikoff and his colleagues have prepared potent lipotropic extracts from pancreatic juice as well as from whole gland (22, 21, 14, 27, 15). Chaikoff and Best now feel that part of the lipotropic effect of the pancreas is the result of its ability to facilitate the utilization of protein. The hydrolysis of protein is believed to make available lipotropic amino acids such as methionine which, by contributing its labile methyl group, enhances the synthesis of choline (3, 6, 7).

The picture which is observed in the diabetic dogs maintained on insulin is an interesting one, regardless of its pathogenesis. In the acutely diabetic animal, severe lipemia is seen accompanied by lipoid deposits in the liver. With the initiation of insulin therapy, the blood lipids drop to abnormally low values and the initial fatty liver disappears. As insulin treatment continues, the animals become extremely insulin-sensitive, so that only small amounts are required for the maintenance of normal blood sugar. During this period the fatty liver reappears The animals become weak, lose weight, and sometimes develop ascites. With the administration of lipocaic or pancreas, there occurs, along with the mobilization of liver fat, elevation of the blood lipids and a considerable increase in the insulin requirement.

The place of lipotropic agents in the clinical management of diabetes is unsettled. Very favorable results have been reported following the administration of lipocaic for certain cases of hepatomegaly, particularly that which is occasionally associated with diabetes in children (5, 17, 33). Following total pancreatectomy in human patients it has so far not been found necessary to administer lipotropic substance to prevent fatty liver.

ARTERIOSCLEROSIS

The problem of degenerative vascular disease is the most significant one which faces the clinician and investigator in the field of diabetes in the insulin era. No explanation is available for the incidence and the premature occurrence of arterial changes in the diabetic. Vascular complications are not related to the severity of diabetes, but probably to its duration (2, 28). Although it is felt by some that proper use of insulin and dietary management can influence vascular degeneration (30), the availability of insulin has thus far only served to exaggerate the incidence of vascular disease of the extremities and coronary arteries by reducing the number of deaths from diabetic coma.

Unfortunately very few data are available concerning the relationship of this complication to experimental diabetes. Dragstedt, in the course of his lipocaic studies, found an incidence of 15% of aortic atherosclerosis in pancreatectomized dogs maintained on insulin (11, 10). This finding was very significant in view of the negligible incidence of such lesions occurring naturally in the domestic dog. The observation was also surprising in view of the low blood cholesterol and total lipid values of pancreatectomized-insulin-treated dogs. Hayes, using cholesterol-fed rabbits which readily develop atherosclerosis, found that the administration of a pancreatic lipotropic extract offered a significant degree of protection against atherosclerotic changes (19) This protection was effective despite the fact that the extract caused no reduction in the hypercholesterolemia which cholesterol-fed rabbits regularly exhibit Further research on the relationship of the pancreas to experimental arterial disease would seem eminently worthwhile.

ABSORPTION OF FATS

In 1904 Lombroso postulated that the pancreas exerted an endocrine control over the absorption of fats from the

intestine (26). This theory was proposed on the basis of his own observations and those of previous investigators who found that total pancreatectomy produced a far greater defect in lipid absorption than did ligation of the ducts or other methods by which the external secretion of the pancreas was excluded from the intestinal tract (23, 24, 25, 35, 34). Subsequent reports differed somewhat on this matter, but numerous results similar to those of Lombroso were reported. This subject was reviewed in more recent times by Coffey (9). It was his finding that ligature and evulsion of the pancreatic ducts in dogs produced a much milder defect in fat absorption than did pancreatectomy when studied shortly after the operation. Absorption became more impaired, as time went on, however, and this impairment was seemingly related to the sclerosis and atrophy of the pancreas which gradually took place following the operation. The establishment of an external pancreatic fistula, on the other hand, caused an immediate defect in absorption comparable to that of pancreatectomy.

The authors suggest that the most likely explanation for the relatively normal digestion during the early period following duct ligation is the entrance of small amounts of external secretion through very fine channels into the intestine These presumably become closed off during the ensuing sclerosis of the acinar tissue. In view of the severely impaired function which is associated with a total external pancreatic fistula, any endocrine mechanism involved in this process seems unlikely.

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CHAPTER VI

THE HYPERGLYCEMIC FACTOR

A considerable degree of attention has been directed in recent months to the possibility of a hyperglycemic or "anti-insulin" factor of pancreatic origin. The interest in a second pancreatic factor has been stimulated by the fact that, whereas the beta cells of the islets of Langerhans are now definitely proven to be the source of insulin, no specific function has been attributable to the alpha cells. Though the alpha cells, having cytoplasmic granules, appear to be secretory elements, *they differ cytochemically from the beta cells.* Evidence from alloxan diabetes indicates, moreover, that they are not merely immature or transitional forms of beta cells, since they are incapable of regenerating beta cells when the latter are chemically destroyed.

The evidence for a hyperglycemic factor as a true internal secretion is not complete but is suggestive, and the presence of such a substance would explain numerous experimental and clinical observations which at present are puzzling. There are two main lines of evidence pointing to the existence of such a factor: first, observations of a paradoxical nature with regard to certain types of experimental and clinical diabetes, and second, the finding of pancreatic extracts with hyperglycemic activity.

EXPERIMENTAL DIABETES

Pituitary (metahypophyseal) diabetes: The metabolic observations on pituitary-diabetic dogs are of particular interest in this connection. Following Houssay's demonstra-

in depancreatized and duct-ligated dogs by the daily feeding of fresh pancreatic juice, *J. Biol. Chem.*, *137:*693 (1941).

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When insulin was withdrawn, however, the pancreatectomized animals passed rapidly into ketosis and coma. Figure 1 illustrates the ketonuria, glycosuria, and insulin requirement of an alloxan-diabetic dog before and after pancreatec-

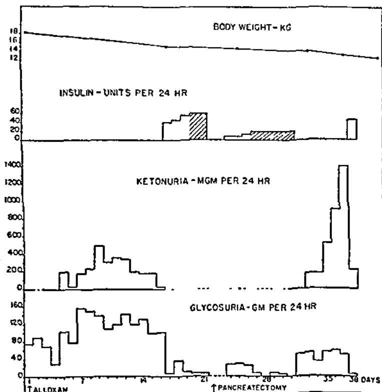


FIG 1 Course of glycosuria, ketosis, and insulin dosage in alloxan-diabetic dog subjected to pancreatectomy. Cross-hatched areas indicate period when insulin control has been established (Thorogood and Zimmerman (63))

tomy. These findings were confirmed by Rodriguez-Candela and co-workers (54). Figure 2 is taken from later studies concerned with the blood ketone levels in these two types of preparations and illustrates the mounting ketosis which

tion that the pituitary was an antagonist to the pancreas in the regulation of the blood sugar, it was found by Young that diabetes could be produced by injection of pituitary extract (29, 68). If daily injections were given over a sufficient period the diabetes became permanent. It was subsequently shown that permanent diabetes thus produced was related to degranulation and atrophy of the beta cells of the islets (51, 27). Certain observations with regard to the metabolism of these animals are significant (70, 69, 36). It was reported by Young and his co-workers that some of these dogs require more insulin for control of diabetes than do pancreatectomized dogs, but that they are able to survive for long periods without insulin treatment. They did not progress rapidly into ketosis and coma as do pancreatectomized dogs untreated. Removal of the pancreas from one such animal resulted in a slight fall in insulin requirement.

Partial pancreatectomy: A similarly paradoxical situation was reported by Dragstedt, who studied the insulin requirement of dogs with 90-94% pancreatectomies (15). These animals required a great deal more insulin for control of glycosuria than is needed by totally depancreatized dogs.

Alloxan diabetes: Goldner and Gomori, who first produced alloxan diabetes in the dog, and Dragstedt reported rather high insulin requirements in these animals (25, 15). Thorogood and Zimmermann studied the glycosuria, ketonuria, and insulin requirements of alloxan-diabetic dogs (63) After these studies had been made, some of the same dogs were subjected to ligation of the pancreatic ducts and others to pancreatectomy. Although the insulin requirement was found to be very high in the alloxan-diabetic animals, it was also observed that they could survive for long periods without insulin and that they displayed very little tendency toward the development of ketosis. Ligation of the pancreatic ducts influenced this situation very little, whereas pancreatectomy resulted in significant diminution of the insulin requirement.

When insulin was withdrawn, however, the pancreatectomized animals passed rapidly into ketosis and coma. Figure 1 illustrates the ketonuria, glycosuria, and insulin requirement of an alloxan-diabetic dog before and after pancreatec-

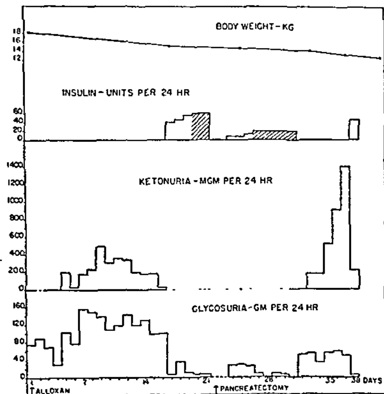


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tomy. These findings were confirmed by Rodriguez-Candela and co-workers (54). Figure 2 is taken from later studies concerned with the blood ketone levels in these two types of preparations and illustrates the mounting ketosis which

is characteristic of pancreatectomized dogs as compared to the low, constant levels that are seen in alloxan-treated animals when insulin is withheld (71). The suggestion was made that the discrepancy between alloxan-diabetes and pancreatectomy in dogs might be explained by a second pancreatic factor, *the source of which is not attacked by alloxan*, but is removed by pancreatectomy. Since alloxan in the dog is deleterious to the beta cells only (25), it is possible that

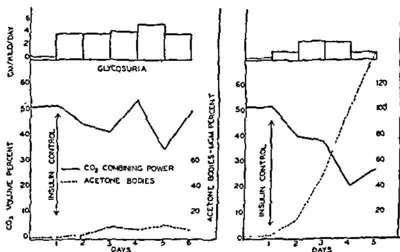


FIG. 2. Glycosuria, ketonemia, and carbon dioxide combining power following withdrawal of insulin treatment in alloxan-diabetic, pancreatic duct-ligated dog (left) and depancreatized dog (right).

the alpha cells could represent this source. The hypothetical substance is contrary to insulin in its action on the blood sugar, but in some way prevents ketosis in the insulin-deficient animal. The existence of such a factor might also explain the findings in Young's pituitary diabetes, another instance of pure beta cell deficiency, and possibly the paradoxical situation described by Dragstedt in connection with *partial pancreatectomy*.

Comparative diabetes: Implications similar to those of the foregoing experiments exist in the findings relative to pancreatectomy in certain species. As was mentioned in Chapter III, it was fortunate that von Mering and Minkowski chose the dog for their classical experiments. Claude Bernard had previously performed pancreatectomy in birds, and, though severe inanition and death resulted, no anomaly of carbohydrate metabolism was demonstrated (2). Many subsequent investigators have confirmed the fact that, with the exception of the owl, no permanent hyperglycemia develops following pancreatectomy in birds (65, 56, 57, 59, 42, 44). The discrepancy in the case of the owl is believed to be related to its carnivorous habits.

In pigs, pancreatectomy produces little or no hyperglycemia but severe ketosis, whereas, in goats the operation is followed by neither hyperglycemia nor ketosis (32, 33, 40). In monkeys, it has been claimed that pancreatectomy produces an unusual picture characterized only by alimentary hyperglycemia and severe fasting hypoglycemia (13, 10). Mirsky has denied this and shown that pancreatectomy in this species produces characteristic hyperglycemia, glycosuria, and ketosis (41).

In those animals in which pancreatectomy produces no hyperglycemia, it is conceivable, though by no means the only possible explanation, that two opposing factors have been removed. This would leave the metabolism in a "neutralized" status similar to that of the "Houssay animal." Unfortunately, adequate comparative studies on alloxan diabetes in these species are not available. Alloxan apparently does produce hyperglycemia in pigeons, but the disease is complicated by severe uricemia, and the animals rapidly die so that no studies of a chronic nature can be done (26, 55). Permanent hyperglycemia has been produced in the monkey by alloxan (1). Alloxan diabetes in the pig and

goat would be very interesting. The present author is not aware of any such studies.

CLINICAL CONSIDERATIONS

Spontaneous diabetes: Certain clinical facts also suggest that more than one endocrine factor may be involved in the genesis of human diabetes. It is recognized for example that some diabetics exhibit extremely high insulin requirements, but do not readily develop ketosis in the absence of treatment. Others with low insulin requirements may be subject to frequent bouts of acidosis and coma. There seems to be little relationship between the extent of the defect in carbohydrate metabolism and the susceptibility to ketosis. This suggests that the two phenomena may be controlled by separate factors.

Pancreatectomy in the human: Of particular interest is the insulin requirement of the totally pancreatectomized human being (53, 49, 4, 52, 14, 67, 64, 19, 50). In general the insulin requirement of such patients is low, in almost all cases ranging between 30 and 40 units per day. The requirements of insulin for these individuals are lower than that seen in most instances of naturally-occurring diabetes, despite the fact that all of the insulin-producing tissue has been removed. Two cases of total pancreatectomy have been reported in the presence of spontaneous diabetes (14, 52). In one case the insulin requirement remained about the same, and in the second it was somewhat reduced It would of course be extremely interesting to know the results of pancreatectomy in highly insulin-resistant diabetic patients. No such cases have been recorded

Despite their low insulin requirements, pancreatectomized human beings do develop ketosis In general, ketosis and acidosis have been mild during the brief periods in which such patients have been studied without insulin (14, 64).

One case, reported by Brunschwig, however, died of diabetic coma following withdrawal of insulin (29).

Data are not available from which to compare alloxan diabetes with total pancreatectomy in man. The question of whether the human islets are sensitive to the necrotizing action of alloxan is still disputed.

Spontaneous hypoglycemia: That the deficiency of an alpha-cell hormone may give rise to characteristic clinical picture was suggested in the recent report of McQuarrie, Bell, Zimmermann, and Wright concerning two cases of familial hypoglycemia in which differential stains of pancreatic tissue revealed alpha cells to be almost entirely absent (39). Severe reduction in the number of beta granules was also observed, however, in these cases

HYPERGLYCEMIC MATERIAL OF PANCREATIC ORIGIN

Hyperglycemic substances in insulin and other pancreatic extracts: Shortly after the discovery of insulin it was observed that extracts of pancreas possessed hyperglycemic as well as hypoglycemic activity. In the early experiments with insulin, it was observed that a short period of hyperglycemia preceded the fall in blood sugar subsequent to insulin administration (35, 12, 20, 38). Murlin and his coworkers were interested in this hyperglycemic phase of insulin activity and were able to prepare by extraction of pancreas insulin-free preparations which possessed the capacity to raise the blood sugar (43, 31, 24). Murlin gave the name of "glucagon" to substances produced by pancreas and other tissues which demonstrated hyperglycemic activity. Subsequently a great many investigators reported the preparation of hyperglycemic materials from the pancreas (22, 37, 34, 7, 28).

Bürger and his colleagues between 1928 and 1937 re-

ported extensive investigations of the hyperglycemic component of insulin (5, 9, 7, 6). They showed that the primary hyperglycemia associated with insulin administration was the result of a second factor similar to insulin and chemically closely related. This factor they did not find in crystalline preparations of insulin, and it was resistant to chemical procedures which destroyed the hypoglycemic activity of insulin, such as boiling with alkali (7, 9). Bürger found, as did Collens and Murlin, that the hyperglycemic action was more profound when the preparations were administered into the portal vein (6, 11). From this he concluded that the material acted to cause release of liver glycogen as glucose into the blood stream. He demonstrated that this glycogenolytic action did not require the presence of the adrenal and was thus not mediated by epinephrine (8).

Two very striking examples of the hyperglycemic-glycogenolytic action of insulin deserve particular mention. Bridge found that if insulin were added to a continuous infusion of glucose (1 unit per gram of glucose), greater and more prolonged hyperglycemia was observed than when glucose alone was administered (3). Equally paradoxical was the finding of Jourdonais and Bruger who observed that when massive doses of insulin (1000 units) were given intravenously, severe hyperglycemia ensued and lasted as long as 190 minutes (30). This elevation of the blood sugar obtained even while some of the animals displayed convulsions.

The separability of hyperglycemic principle from insulin: *To decide whether hyperglycemic activity was a* property of the insulin molecule itself or actually a separate substance, Geiling and de Lawder studied in detail the blood sugar response of crystalline insulin prepared by the method of Abel (23) They were unable to associate any hyperglycemic phase with the action of the crystalline product though they did observe hyperglycemia following the injection of various fractions which were discarded in the process

of preparing insulin crystals. Despite the findings of these investigators, later work by Olsen and Klein demonstrated that some primary hyperglycemia was characteristic of the blood sugar response even to pure zinc-insulin crystals (45). Zimmermann and Donovan, using insulin preparations in which hypoglycemic activity was destroyed by treatment

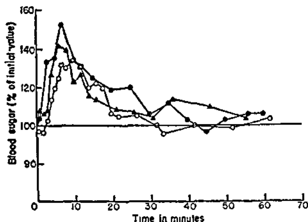


FIG 3. Hyperglycemic response to intravenous injection of 0 1 mgm. per kilogram of cysteine-inactivated amorphous insulin in normal dogs (Zimmermann and Donovan (72))

with cysteine, found approximately equal hyperglycemic activity with material made from LILLY amorphous insulin and LILLY zinc insulin crystals (72) (Figures 3 and 4).

In 1946, however, de Duve and his co-workers made the very important discovery that a Danish insulin preparation called NOVO possessed essentially no hyperglycemic property (16). This fortunate circumstance, the existence of a hyperglycemia-free preparation, has made it possible to study the separate action of insulin itself and as we shall see, has clarified considerably our understanding of at least one of insulin's physiological actions.

The mechanism of action of hyperglycemic factor: Al-

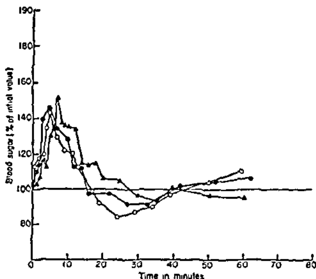
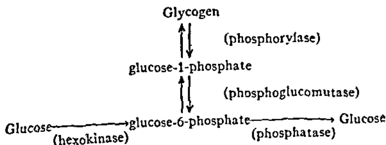


FIG. 4. Hyperglycemic response to intravenous injection of 0.1 mgm. per kilogram of cysteine-inactivated crystalline insulin in normal dogs (Zimmermann and Donovan (72)).

though it was the opinion of most investigators that the hyperglycemic action of pancreatic preparations depended upon their action on liver glycogen, final proof of this mechanism was not available before the *in vitro* experiments of Sutherland and Cori who studied the effect of insulin preparations on glycogen formation in liver slices (60). It will be remembered that the following steps are involved in the equilibrium between glucose and glycogen:



The final step in the formation of glycogen is polymerization of glucose-1-phosphate to glycogen under the influence of the enzyme phosphorylase.

It was found by Sutherland and Cori that when ordinary insulin preparations, either amorphous or crystalline, were added to this system *in vitro*, glucose was rapidly formed from glycogen, and phosphorylase activity was decreased. The same was true when cysteine- or alkali-inactivated insulin was added. The addition of the hyperglycemia-free NOVO insulin, on the contrary, stimulated no glycogenolytic activity. From these experiments the direct action of the hyperglycemic fraction on the liver glycogen stores is clear.

A further *in vivo* demonstration of the fact that the hyperglycemic principle acts only on the liver glycogen is shown in Figure 5. Because of the absence of ketosis in alloxan-diabetic dogs, Zimmermann and Donovan were interested to find out whether the hyperglycemic factor prepared by cysteine inactivation of commercial insulin would reduce ketosis in pancreatectomized dogs (72). It was found that the reverse was the case. As can be seen in the curves of blood sugar and ketone bodies following the administration of the hyperglycemic material, the blood ketone levels always rose with the blood sugar and in fact followed the variations in the latter rather closely. Similar changes were found during the primary hyperglycemic phase following the injection of intact (non-inactivated) insulin (Figure 6). This finding seems consistent with the theory that the blood sugar is being elevated at the expense of liver glycogen, the depletion of which is rapidly reflected in the increase in blood ketone levels.

Pincus provided further evidence for the direct effect on the liver by showing that the hepatectomized animal exhibited no response to hyperglycemic extracts (48, 47).

Another question which arises is whether the hypergly-

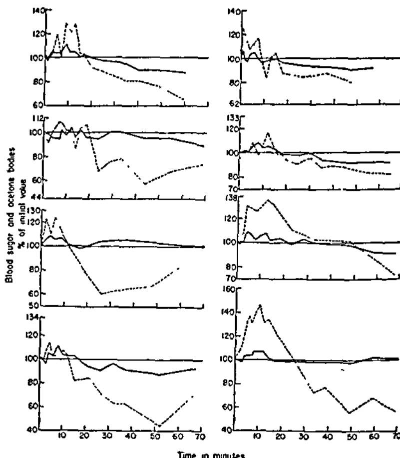


FIG. 5. Blood sugar and acetone-body levels in depancreatized dogs following intravenous injection of cysteine-inactivated amorphous insulin (Solid line· *sugar;* broken line. *acetone bodies*) (Zimmermann and Donovan (72))

cemic factor produces its effect by some sort of direct antagonism to insulin. Zimmermann and Donovan were unable to demonstrate any protective effect against insulin-induced convulsions associated with the subcutaneous administration of hyperglycemic factor to mice (72). Pincus,

however, has shown that intravenous administration of the material can prevent the hypoglycemia from small doses of insulin, and as Levine and his co-workers point out, it may not be valid to draw conclusions from the action of subcutaneously injected material since its activity is rapidly destroyed by the tissues (46, 66).

The source of the hyperglycemic principle: Sutherland and de Duve found that the glycogenolytic factor could be found in the pancreas and in the upper portion of the gastric mucosa of the dog (62, 61). It was not recovered from other tissues. It was found in fetal calf pancreas (in which the acinar tissue is not developed) and in the fibrosed pancreas of animals in which the pancreatic ducts were ligated. It was also found in normal amounts in the pancreas of alloxan-treated rabbits. Extracts from alloxan-treated rabbits were found to cause prolonged hyperglycemia when injected into

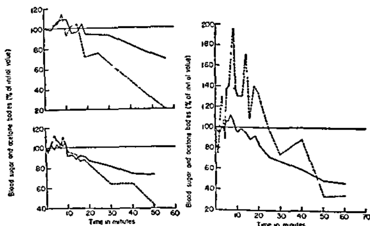


Fig 6 Blood sugar and acetone-body levels in depancreatized dogs following intravenous injection of intact amorphous insulin. (Solid line *sugar*, broken line. *acetone bodies*) (Zimmermann and Donovan (72))

normal rabbits with no subsequent hypoglycemic phase. From these data the authors concluded that the alpha cells of the pancreas were the most likely source of the hyperglycemic-glycogenolytic factor. Confirmatory evidence for this came from the work of Pincus who found hyperglycemic material in the pancreas of alloxan-treated dogs, but not from hog or steer salivary glands (48).

Physiological production of hyperglycemic substance: Sutherland and Cori were unable to find hyperglycemic material in the blood from the pancreato-duodenal vein or perfusates from isolated pancreas (61). Foà and co-workers, on the other hand, found that in cross-circulation experiments in which the recipient dog was transfused from the pancreato-duodenal vein of an alloxan-diabetic donor, the injection of glucose in the donor dog caused a rise in blood sugar of the recipient (21). This rise was greater than that which occurred when the recipient was transfused from a mesenteric vein This is the only experiment which has been reported which demonstrated acutely the *in vivo* production of hyperglycemia by the pancreas. If this represents a true hormonal activity, it is difficult, on a teleological basis, to understand why such a response would be elicited following the injection of glucose as a stimulus, unless perhaps, it might be released along with insulin as a mechanism for attenuating the action of the latter

The significance of "hyperglycemic factor": We must await more evidence to decide whether this factor which is recovered in pancreatic extracts is truly an alpha-cell hormone or an endocrine substance at all. That its presence or absence plays a role in certain diseases is an inviting hypothesis, but not entirely proven.

On the other hand, the presence of a blood sugar-elevating principle in insulin preparations does reconcile some of the previously-reported conflicting data on the action of insulin.

In Chapter III the confusing question of the relationship of insulin to the liver glycogen was discussed. It was difficult to understand why, if insulin repletes the liver glycogen and reduces ketosis in the diabetic animal, it consistently promotes glycogenolysis in the normal liver. It is now evident that glycogenolysis is not a function of insulin but of a second factor which has been regularly administered along with it. The experiments of Sutherland and Cori give adequate explanation for results such as those reported by Shipley and Hümel who found that insulin stimulated glycogenolysis in glycogen-rich liver slices (58). Evans, who previously reported that insulin prevented glycogenesis even in animals rendered hyperglycemic with glucose (17), now reports that no such effect is observed with NOVO insulin (18).

The findings of McQuarrie *et al.* with regard to the paucity of alpha cells in two cases of congenital hypoglycemia have been mentioned (39). Further confirmation of the association of abnormalities of the alpha cells with clinical disease would be of great interest.

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CHAPTER VII

CONCLUSIONS

FROM the physiologist's viewpoint the most significant goal for future research in this field would seem to be the elucidation of the chemical mechanism by which insulin exerts its metabolic action. To date no single metabolic reaction has been found to be influenced by insulin in such a way that such influence could account for the over-all physiologic activity of the hormone. Even though no precise chemical explanation for the action of insulin has been reached, however, it is apparent that this goal has been more nearly approached for insulin than for any other hormone. The solution of the question will therefore represent definitive advance for the science of endocrinology.

The clinician's goal is the understanding of the endocrine functions of the pancreas to the extent that all the clinical manifestations of diabetes and perhaps other metabolic disorders can be corrected. Although the discovery of insulin has been one of the most dramatic scientific achievements of our time, one must admit that the treatment of diabetes is still not completely satisfactory Certain aspects of clinical diabetes are baffling. The phenomenon of insulin resistance, for example, is of great theoretical and practical importance. Why do many diabetics require more insulin than the totally depancreatized human being? Does the factor concerned with insulin resistance reside in the pituitary, the adrenal, or perhaps in the pancreas itself? Of even greater importance and equally poorly understood are the degenerative complications of clinical diabetes. In what way are premature vascular changes related to the metabolic impairment produced by the

absence of insulin, and is insulin the only factor involved in their prevention?

It seems likely that the considerations which were presented in Chapter VI may eventually resolve the confusion which has interrupted progress in certain directions. The existence of more than one pancreatic hormone is an inviting hypothesis, but one that must await further evidence, particularly evidence of a clinical sort. Regardless of whether such a hormone exists or not, the experimental data which have suggested its presence are of themselves important. It is of particular moment that various types of experimental diabetes do not present identical metabolic appearances. Historically the depancreatized dog has been the source of the largest body of experimental evidence. Alloxan diabetes differs in some respects, and because of the anatomical characteristics of the lesion produced by this drug we must assume that alloxan creates a situation as nearly as possible related to pure insulin deficiency. The older experimental literature spoke frequently of the "totally diabetic animal." We must now confess that we do not know the meaning of "total diabetes." Clinical experience had indicated all along that there was no justification for such a concept.

As distinguishing between different types of experimental preparations may offer greater insight into the precise metabolic deficiency, so should the discovery that ordinary insulin preparations are not homogeneous As we have already seen, this knowledge has clarified greatly the concept of insulin's regulation of the liver glycogen

Whether this idea of the dual nature of pancreatic endocrine function will help to explain such phenomena as insulin resistance and the degenerative complications of diabetes remains for the future to decide.

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